Screening of endophytic Streptomycetes isolated from *Parthenium hysterophorus* L. against nosocomial pathogens

Rabia Tanvir*, Imran Sajid and Shahida Hasnain
Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

Abstract: *Parthenium hysterophorus* L. is an obnoxious weed of the family asteraceae recognized for its detrimental effects and significant economic losses to agriculture. In this study 42 endophytic streptomycetes strains were isolated from its roots and leaves. The isolates were identified by morphological, microscopic, biochemical and physiological characterization as members of genus *Streptomyces*. In 16S rRNA gene sequencing the selected isolates exhibited maximum similarity with *Streptomyces rochei* (99%), *Streptomyces litmocidini* (99%), *Streptomyces enissocaesili* (99%), *Streptomyces djakartensis* (99%), *Streptomyces olivaceus* (99%), *Streptomyces spp* (99%), *Streptomyces plicatus* (99%), *Streptomyces geysiriensis* (99%) and *Streptomyces vinaceusdrappus* (99%). In biological screening the crude extracts of 12 strains exhibited significant antimicrobial activity against multi drug resistant nosocomial pathogens including *Pseudomonas, Enterobacter, Bacillus, Escherichia coli, Staphlococcus aureus* and *Candida albicans*. In chemical screening by Thin Layer Chromatography (TLC) the extracts exhibited an impressive diversity of the bioactive secondary metabolites. Additionally High Performance Liquid Chromatography (HPLC-UV) chromatographs revealed many impressive peaks of unidentified bioactive metabolites. As such this is a first study reporting the isolation, identification and screening of endophytic *Streptomyces* from the invasive weed. The results provide an insight into an untapped endophytic environment yet to be explored which might be a promising source of lead antimicrobial agents.

Keywords: Endophytes, Streptomycetes, *Parthenium hysterophorus* L, nosocomial pathogens

INTRODUCTION

To provide support and assistance in all aspects of the human conditions, there is an ever growing need for new and useful compounds due to the emergence of life threatening viruses, multidrug resistant bacteria; persistent problems of disease in people undergone organ transplantation, and the remarkable augmentation of fungal infections in the world (Strobel and Daisy, 2003). The bioactive microbial secondary metabolites still remain the most important source for discovery of new and potential drugs (Firáková *et al*., 2007). In this context the microorganisms of unique and unexplored ecological niches such as deserts, forests, marine ecosystems and most prominently endophytes are considered as an attractive source of new compounds. Nearly all plants retain a microbiota and some microbial colonizers take up residence in the inner tissues of plants as endophytes (Bascom-Slack *et al*., 2009). These endophytes produce a plethora of substances including antimycotics, anticancer compounds, novel antibiotics, immunosuppressants (Strobel *et al*., 2004) and a wide variety of unique structured bioactive secondary metabolites comprising benzopyranones, alkaloids, flavonoids, phenolic acids, chinones, steroids, quinones, tetralones, xanthones, terpenoids, which provide survival conditions and defense to their host plant (Pimentel *et al*., 2011) and are a fertile source of pharmacologically active natural products with probable agrochemical or medicinal applications (Ding *et al*., 2010).

**Corresponding author:* e-mail: shahida@mmg.pu.edu.pk

Streptomycetes are described by their capability for producing a wide variety of pharmacologically active substances (Alam *et al*., 2010) about 80% of the therapeutically useful antibiotics, i.e., aminoglycosides; cephamycins, polyenes, tetracyclines, macrolides, benzopyrones and triazolopyrimidine are produced by them (El-Gendy and EL-Bondkly, 2010). These are mostly found living as saprophytes in the soil, but lately some species have been described in plant tissue and the rhizosphere of plant roots (Alam *et al*., 2010). It is now apparent that these filamentous bacteria also occur in living tissues of certain higher plants as endophytes and may serve as sources of novel bioactive compounds, as majority of them are untapped (Zin *et al*., 2007).

Parthenium hysterophorus L. a herbaceous plant native of tropical and subtropical America (Romero *et al*., 2001) is an annual ephemeral herb of neo-tropical origin which now has a pan-tropical distribution (Khan *et al*., 2010). It has shown significant adaptiveness in eastern and south Africa, southern USA, Pakistan, Bangladesh, Nepal, Southern China, and Vietnam. In India, Ethiopia, Australia and Pakistan it has become a serious weed in agriculture posing significant economic losses, as of this reputation, it is documented in top ten weeds in the world (Shabbir and Javaid, 2010). The weed has not only become naturalized during the last 50 years but also has spread at an alarming rate becoming a prevailing and a problematic weed of crops/wastelands in numerous parts of world. In the mid-1950s it is thought to have been unintentionally introduced to bordering country India and

since then has extended over most parts of Pakistan mainly in Punjab, NWFP and Kashmir area. The major reason behind its ability to become dominant is its high reproductive potential and fast growth rate (Javaid *et al*., 2010). It is reported to be widely distributed in the plains of Punjab including district Lahore, Sialkot, Sheikhupura and Okara (Riaz and Javaid, 2009). Allelopathic interference is considered one of the vital mechanisms for the triumphant founding of invasive exotic weeds (Maharjan *et al*., 2007). *P. hysterophorus* L. is reported to have an allelopathic effect such as the ability to develop and reproduce in a wide range of conditions (Tamado and Milberg, 2000), creating tough competitiveness for nutrients and soil moisture (Shabbir and Javaid, 2010). Its toxic effects have been reported on many agricultural crops (Knox *et al*., 2010), animal husbandry, human health, crop production and biodiversity of native flora (Khan *et al*., 2010). It is therefore the contributory mean of serious health, environmental and economic problems (Shabbir and Javaid, 2010).

The aim of the present study was to isolate endophytic streptomycetes from *P. hysterophorus* L. and to screen their potential for the production of bioactive secondary metabolites. Endophytic bacteria have been isolated and studied from nearly every wild, herbal and medicinal plant, cultivated crops, even, trees, and pasture species but according to the best of our knowledge no study has been carried out on endophytic streptomycetes from *P. hysterophorus* L. which is known for its harmful aspects only.

MATERIALS AND METHODS

Sample collection

Healthy plants of *Parthenium hysterophorus* were collected from the agricultural lands at the university campus (latitude: 31° 29'43.98 N, longitude: 74° 17'44.55 S, elevation: 711ft.) during the period of June 2009 to November, 2009. The plants were carefully rooted keeping the roots and leaves intact and the samples were shifted to the lab in labeled sterile bags and were processed within 4 hours after collection.

Isolation and identification of endophytic streptomycetes

Each of the plant sample was thoroughly washed under running tap water to remove soil particles, then the plant tissues were divided into 0.5 cm segments and were surface sterilized by the five step sequential process; first the segmented tissues were immersed in 70% ethanol for five minutes and then in 0.9% sodium hypochlorite solution for twenty minutes. Each of the tissue was washed three times with autoclaved distilled water to completely remove the disinfectants, after washing the tissues were dipped in 10%$NaHCO_3$ for ten minutes for disruption and for inhibition of endophytic fungal growth. Finally the tissue segments were washed with autoclaved distilled water.

The surface sterilized tissue segments were plated on actinomycetes isolation agar (Difco laboratories), Glycerol casein KNO_3 agar (Kuster and Williams, 1964) and Rice agar (rice boiled for 30 min, then filtered to remove insoluble materials, 18.0 g/L agar was added, pH 7.6) and were incubated at 28ºC for a period of 3 weeks. As a negative control, plant tissue segments were aseptically rolled and plated on the same three media and the plates were incubated at 28ºC to ensure the surface sterilization. The selected colonies were purified by repeated sub culturing on glucose yeast extract malt extract agar (GYM) (Shirling and Gottlieb, 1966) until pure cultures were obtained. The isolates were identified on the basis of morphological, biochemical and physiological characteristics and 16S rRNA gene sequencing. Biochemical and, physiological characterization such as melanin production, utilization of nine different sugars as carbon source was performed according to the "conventional" methods (Shirling and Gottlieb, 1966). Formation as well as utilization of organic acids and oxalate, hydrolysis of esculin, arbutin, urea and allantoin was also determined. 16S rRNA gene sequencing was done commercially at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea) to determine the final taxonomic status. The gene sequence data obtained was first analyzed using the advanced BLAST search program at the NCBI website: http://www.ncbi.nlm.nih.gov/BLAST/. The nucleotide sequence data was deposited to GenBank, and the Genbank accession numbers for 12 strains were obtained (table 2).

Determination of antimicrobial activity

For the detection of bioactive secondary metabolites produced by these *Streptomyces* isolates, agar diffusion method was used. The isolates were inoculated in 30ml of glucose yeast extract malt extract (GYM) broth (Shirling and Gottlieb, 1966) and were incubated at 28°C on a shaker at 95 rpm for 5-7 days. 1 ml of cell free supernatant was centrifuged at 10,000 rpm for few minutes. The plates for the test organisms were prepared by pouring 14ml of Luria Bertani agar (LB-agar) (Gerhardt, 1994) as base layer; after solidifying, this was overlaid with 4ml of inoculated seed layer. Agar wells (5 mm diameter) were made by using sterile cork borer and 60µl of the supernatant was loaded to these wells, the plates were left at room temperature for 2 hours for the supernatant to diffuse and were incubated at 37°C for 18-24 hours. After incubation zones of inhibition were measured in mm.

Preparation of crude extracts

The isolates were cultivated on small scale, 150ml of glucose yeast extract malt extract (GYM) broth (Shirling and Gottlieb, 1966) was inoculated with the *Streptomyces*

strains and incubated at 28°C on a linear shaker (Sanyo orbital shaker incubator, Japan) at 95 rpm for 5-7 days. After the incubation was completed the culture broth was harvested and 150 ml of ethyl acetate (1:1) was added and the cells were subjected to ultra-sonication in a sonicator (Ultrasona Medi-II, J.P Selecta S.A) for 15-30 minutes. After sonication the mixture were added to a separating funnel to divide the content into two distinct layers. The upper layer of ethyl acetate was carefully separated and evaporated on a rotary evaporator (Laborta 4000 efficient, Heildolph Germany) to get the crude extracts. The final dried extracts were re suspended in 1-5ml of methanol and were kept at 4°C. These extracts were analyzed by thin layer chromatography (TLC) and by high performance liquid chromatography (HPLC-UV).

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was carried out as described previously by Kirchner (Kirchner, 1978). In this method a small drop of sample was spotted onto the TLC plate (TLC Aluminum sheets 20×20 Silica Gel 60 F_{254} Merck, Germany) with a capillary and was air dried; the spotting process was repeated by superimposing more drops on the original spot for obtaining appropriate quantity (2-5µg) of the sample on the plate. The TLC plates were developed with a 10% MeOH/ CH_2Cl_2solvent system. The TLC plates were visualized under UV light (254 nm and 366 nm) and the components showing UV absorbance or fluorescence were scanned and documented. Later the TLC plates were sprayed with Ehrlich's reagent and Anisaldehyde /H_2SO_4 reagent for further localization of interesting compounds. The colored bands produced by the reaction between spray reagent and the metabolites were marked and were documented by scanning.

High performance liquid chromatography analysis (HPLC-UV)

The extracts were analyzed on the HPLC system (Sykum HPLC system) using the clarity chromatography data system. HPLC system consisted of two pressure pumps (Syknm S1122 delivery system), an injection port with a 20 µl loop (Syknm S 5111 injector valve bracket), and a UV detector (Syknm S 3210 UV/Vis detector). The column used was RP C18 (Thermo Hypersil Keystone, 250 x 4.6 mm 5µm Hypersil). Mobile phase was methanol and water (95:5) and the flow rate was adjusted to 1ml/min. The extracts were first dissolved in 200 µl methanol and 20µl of the sample was injected by a microsyringe. The sample running time was 15 minutes and UV absorbance was determined at 254nm.

RESULTS

A varied number of endophytic *Streptomyces* strains were recovered from different tissues of *P. hysterophorus* (table 1). Majority of the strains exhibited significant antimicrobial activity against nosocomial pathogens with zones of inhibition ranging from 10mm to 25mm (table 3).

The chemical screening of the endophytic streptomycetes carried out by using Ehrlich's reagent for staining revealed purple and red spots. Whereas, staining with Anisaldehyde/H_2SO_4 reagent resulted in red, purple, orange colored spots, as well as dark blue, purple, yellow, brown, red and green spots (fig. 2) which are indication of compounds that are precursor to many pharmaceuticals.

DISCUSSION

Isolation of endophytic streptomycetes from P. hysterophorus tissues

A total number of 42 Streptomyces strains were obtained in the study. The majority (33) of the strains were obtained from the roots probably because a morphologically, physically, and chemically complex microcosm is formed in the root tissues. Such environment provides habitats for diverse microbial populations like bacteria, endophytic fungi and mycorrhizal fungi. These organisms are a source of novel biologically active secondary metabolites (Silvani *et al*., 2008). Since *Streptomyces* mainly reside in the soil where the roots are in direct contact with it therefore any damage to the roots may result in the migration of these *Streptomyces* inside the plant tissue and colonizing there.

Characterization of streptomycetes

All the selected isolates exhibited similar morphological features i.e. the appearance of a dry, rough colony texture with variable colored substrate. The aerial mycelium as well as soluble pigment production was also similar. The physiological characterization was performed following the methods adopted in International *Streptomyces* Project (ISP) Shirling and Gottlieb (Shirling and Gottlieb, 1966). The isolates exhibited a positive test for melanin formation and the ability to utilize nine different sugars as sole carbon source which is distinctive for the genus *Streptomyces.* The BLAST analysis of 16S rRNA gene sequence data of the selected strains showed alignment of these sequences with reported 16S rRNA gene sequences in Genbank. The number of nucleotides sequenced in each case and the gene bank accession numbers for selected strains are summarized in table 2.

Antimicrobial activity of the selected streptomycetes against nosocomial pathogens

Results of the biological screening of the selected endophytic streptomycetes revealed that out of 42 strains 12 strains i.e. RT-6, RT-13, RT-18, RT-36, RT- 46, RT-50, RT- 53, RT-56, RT-57, RT-59, RT-60 and RT- 67 exhibited significant antimicrobial activity against nosocomial pathogens (table 3 and fig. 1). The strain RT-53 showed maximum zone of inhibition of 25mm against

Enterobacter spp. and 20mm against clinical isolate of *S. aureus.* The remaining strains showed zones of inhibitions between 10mm to 25mm which is noteworthy especially where multidrug resistant pathogens are concerned. Our results are in good agreement with a study carried out in China, the endophytic streptomycetes isolates investigated in this study also showed impressive antimicrobial activity with zone of inhibition diameters ranging from 10 mm to more than 15 mm against the clinical isolates of *E. coli* and *S. aureus* (Li *et al*., 2008). Our results are also supported by another study on endophytic actinomycetes from the upper Amazonian rainforest in Peru. All the endophytes in the study also demonstrated strong antimicrobial activity (≥90% growth inhibition) against a minimum of six test organisms including *E. coli* (Bascom-Slack *et al*., 2008). In addition to activity against bacterial pathogens our isolates also showed significant activity against important fungal pathogen *Candida albicans* with zones of inhibition ranging from 10-22mm (fig. 1) which clearly indicated that these isolates are biologically active and can be a promising source for useful antimicrobial agents.

Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC-UV)

The chemical screening of the endophytic streptomycetes was done and the extracts were analyzed through TLC. Using Ehrlich's reagent for staining the colored spots obtained indicated the presence of different functional groups like amines and indole derivatives in the extracts. Indol, an aromatic heterocyclic compound is precursor to many pharmaceuticals along with N-heterocycles. It is a possibility that these endophytic *Streptomyces* extracts may contain these derivatives making them active against a wide variety of bacteria. Staining with Anisaldehyde/H_2SO_4 reagent resulted in a variety of colored spots demonstrating the existence of the functional groups like phenols, steroids, and terpenes in the extracts (fig. 2).

Table 1: Endophytic streptomycetes isolates obtained from *Parthenium hysterophorus*

Plant Sample	Plant segment(s)	Condition of the plant	Isolates
Parthenium hysterophorus	Roots	Fresh	RT-6, RT-7,RT-36-67
	Shoots and leaves	Fresh	RT-10, RT-11
	Slurry of roots, shoots and leaves	Fresh	RT-13, RT-14, RT-15, RT-16, RT-17, RT-18

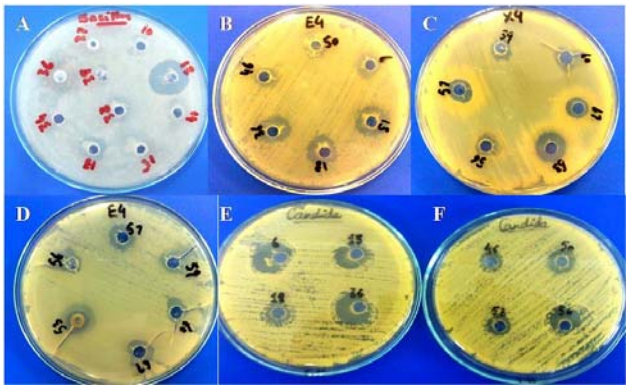

Fig. 1: Antimicrobial activity of the selected endophytic *Streptomyces* against nosocomial pathogens. A Activity of RT-10, RT-13, RT-14, RT-15, RT-18, RT, 34, RT-36, RT-37, RT-38, and RT-39 against *Bacillus*. B Activity of RT-6, RT-13, RT-18, RT-36, RT-46, and RT-50 against *Enterobacter.* C Activity of RT-56, RT-57, RT-59, RT-60, RT-63, and RT-67 against *Pseudomonas.* D Activity of RT-53, RT-56, RT-57, RT-59, RT-60, and RT-67 against *Enterobacter.* E Activity of RT-6, RT-13, RT-18, and RT-36 against *Candida albicans.* F Activity of RT-46, RT-50, RT-53, and RT-56 against *Candida albicans.*

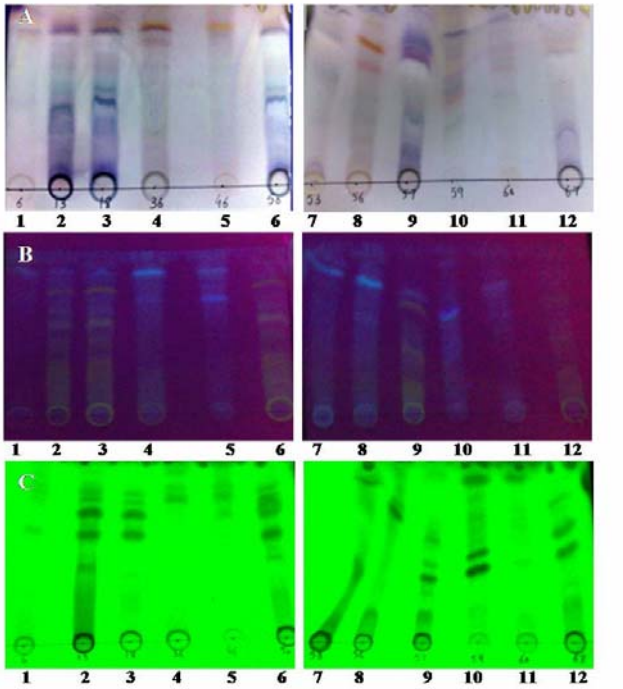

Fig. 2: Chemical screening using TLC detection. TLC plates A) After staining with anisaldehyde/H_2SO_4 reagent, B) under UV at 254 nm, C) under UV at 366 nm. Numbers 1-12: Crude extracts of *Streptomyces* strains 1= RT-6, 2= RT-13, 3= RT-18, 4= RT-36, 5= RT-46, 6= RT-50, 7= RT-53, 8= RT-56, 9= RT-57, 10= RT-59, 11= RT-60, 12= RT-67

Table 2: Results of 16S rRNA gene sequencing of the endophytic streptomycetes

Streptomyces strains	No. of Nucleotides sequenced (bp)	*Streptomyces Spp.*	% Homology	Gen bank accession No.
RT-6	1425	*Streptomyces rochei*	99%	HQ909753
RT-13	1442	*Streptomyces litmocidini*	99%	HQ909754
RT-18	1444	*Streptomyces rochei*	99%	HQ909755
RT-36	1445	*Streptomyces rochei*	99%	HQ909756
RT-46	1445	*Streptomyces enissocaesili*	99%	HQ909757
RT-49	1444	*Streptomyces djakartensis*	99%	HQ909758
RT-54	1445	*Streptomyces olivaceus*	99%	HQ909759
RT-56	1442	*Streptomyces spp.*	99%	HQ909760
RT-57	1445	*Streptomyces plicatus*	99%	HQ909761
RT-63	1444	*Streptomyces geysiriensis*	99%	HQ909762
RT-64	1443	*Streptomyces spp.*	99%	HQ909763
RT-67	1444	*Streptomyces vinaceusdrappus*	99%	HQ909764

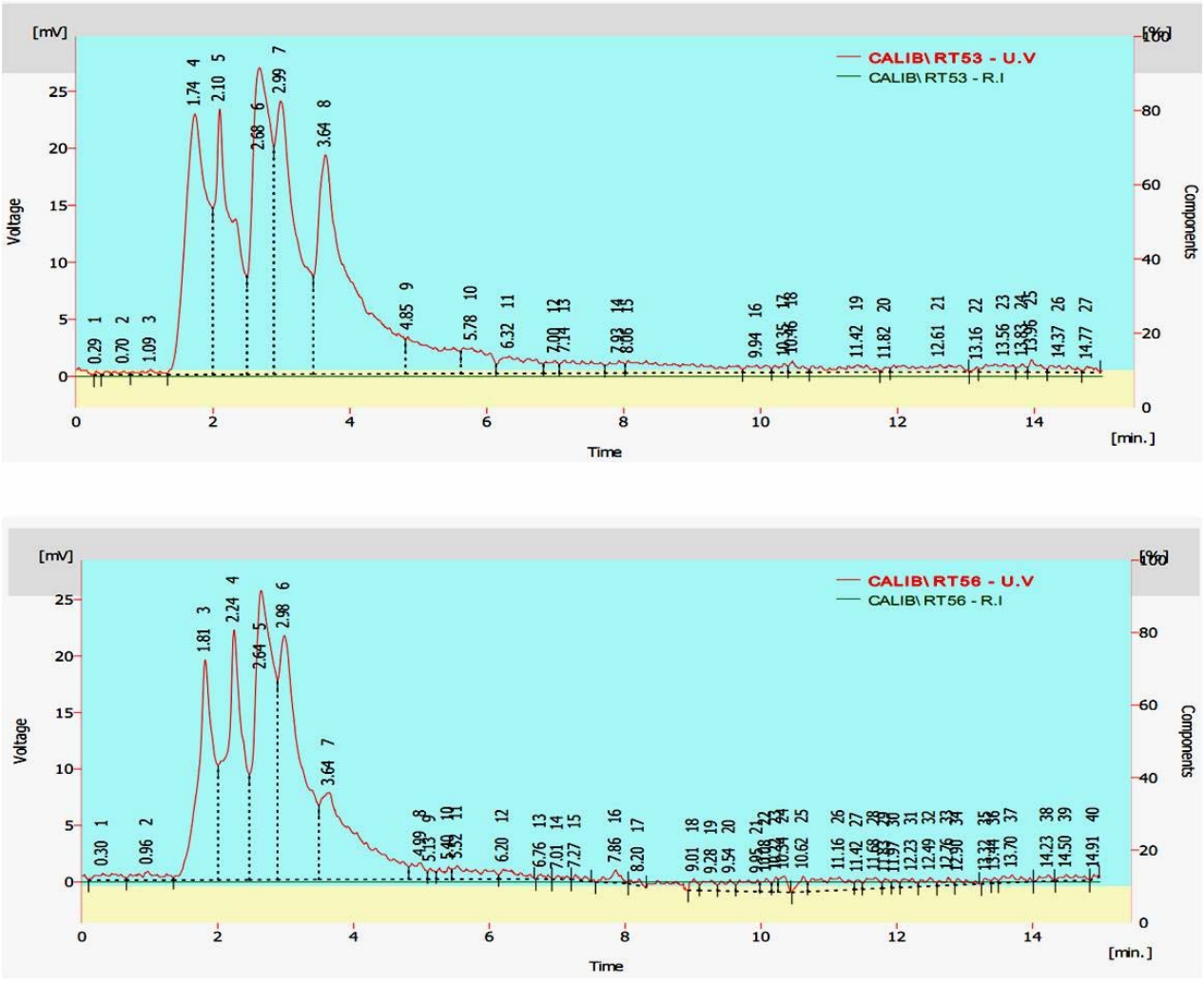

Fig. 3a: HPLC-UV chromatogram of crude extract of the isolate RT-53. **b**: HPLC-UV chromatogram of crude extract of the isolate RT-56.

Table 3: Antimicrobial activity of the endophytic streptomycete isolates against nosocomial pathogens

Strain No.	*Zone of Inhibition (mm)							
	Biofilm formers					Clinical isolates		
	Bacillus	*X4*	*M9*	*S2*	*E4*	*Staph. aureus*	*E. coli*	*C. albicans*
RT-6	18	13	15	18	15	15	15	22
RT-13	20	12	19	16	16	15	13	21
RT-18	18	15	14	18	15	16	15	17
RT-36	15	15	20	15	15	19	15	21
RT-46	20	15	11	12	11	15	14	12
RT-50	17	18	10	10	10	10	10	16
RT-53	18	16	25	14	17	20	15	12
RT-56	20	13	15	10	15	10	15	22
RT-57	17	13	10	10	14	15	10	10
RT-59	15	12	15	10	14	16	10	16
RT-60	16	20	15	10	14	10	10	22
RT-67	15	10	14	10	15	10	10	15

X4, *Pseudomonas spp*.; M9, *Enterobacter; S2, Enterobacter; E4, Enterobacter, Staph. aureus, Staphlococcus aureus; E. coli, Escherichia coli; C. albicans, Candida albicans*

* The zone of inhibition greater than 10mm was considered significant

HPLC-UV chromatograms also revealed peaks of unidentified bioactive compounds at different retention times (t_R). The extracts of the isolate RT-53 gave numerous distinct peaks at variable retention times out of which 5 prominent peaks were at the retention time of 1.74, 2.10, 2.68, 2.99, 3.64 minutes with peak areas (area%) 16.0, 13.9, 16.0, 16.8 and 21.1 respectively (fig. 3A). Similarly the chromatograms of the isolate RT-56 also showed 5 major peaks at retention time of 1.81, 2.24, 2.64, 2.98, and 3.64 minutes with peak areas (area%) of 13.3, 16.2, 21.1, 20.8 and 13.7 respectively (fig. 3B). These chromatograms specify that these unique *Streptomyces* have the ability to produce various bioactive compounds concurrently in good concentrations.

It can be conferred from the above results that the bioactive streptomycetes residing in different tissues of weed *P. hysterophorus* are a promising source of useful compounds. The potential of these streptomycetes can be exploited for future applications by testing their anti parasitic, antiviral, anti tumor and antifungal activities along with the structure elucidation of the bioactive secondary metabolites which may result in the identification of new drugs.

REFERENCES

Alam MT, Merlo ME, Takano E and Breitling R (2010). Genome based phylogenetic analysis of *Streptomyces* and its relatives. *Mol. Phylogenet. Evol.,* **54**: 763-77.

Bascom-Slack CA, Ma C, Moore E, Babbs B, Fenn K, Greene JS, Hann BD, Keehner J, Kelley-Swift EG, Kembaiyan V, Lee SJ, Li P, Light DY, Lin EH, Schorn MA, Vekhter D, Boulanger LA, Hess WM, Vargas PN, Strobel GA and Strobel SA (2009). Multiple, novel biologically active endophytic actinomycetes isolated from upper Amazonian rainforests. *Microbiol. Ecol.,* **58**: 374-383.

Ding L, Münch J, Goerls H, Maier A, Fiebig HH, Lin WH, Hertweck C (2010). Xiamycin: A pentacyclic indolosesquiterpene with selective anti-HIV activity from a bacterial mangrove endophyte. *Bioorg. Med. Chem. Lett.,* **20**: 6685-6687.

El-Gendy MMA and EL-Bondkly AMA (2010). Production and genetic improvement of a novel antimycotic agent, Saadamycin, against Dermatophytes and other clinical fungi from Endophytic *Streptomyces sp*. Hedaya48. *J. Ind. Microbiol. Biotechnol.,* **37**: 831-841.

Firáková S, Šturdíková M and Múčková M (2007). Bioactive secondary metabolites produced by microorganisms associated with plants. *Biologia Bratislava*., **62**(3): 251-257.

Gerhardt P (1994). Methods for General and Molecular Bacteriology. Washington DC, American Society for Microbiology.

Javaid A, Shafique, S, Bajwa, R, shafique S (2010). Parthenium management through aqueous extracts of *Alstonia scholaris. Pak. J. Bot.,* **42**(5): 3651-3657.

Khan MA, Qureshi RA, Gillani SA, Ghufran MA, Batool A and Sultana KN (2010). Invasive species of federal capital area Islamabad, Pakistan. *Pak. J. Bot*., **42**(3): 1529-1534.

Kirchner JG (1978). Techniques of Chemistry in Thin Layer Chromatography. Vol XIV, 2nd ed, Wiley-Interscience, Chichester, UK.

Knox J, Jaggi D and Paul MS (2010). Evaluation of allelopathic potential of selected plant species on *Parthenium hysterophorus. Egypt. J. Biol.*, **12**: 57-64.

Kuster E and Williams ST (1964). Selection of media for the isolation of streptomycetes. *Nature*, **202**: 928-929.

Li J, Zhao GZ, Chen HH, Wang HB, Qin S, Zhu WY, Xu LH, Jiang CL and Li WJ (2008). Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. *Lett. Appl. Microbiol*., **47**: 574-580.

Maharjan S, Shrestha BB and Jha PK (2007). Allelopathic effects of aqueous extract of leaves of *Parthenium hysterophorus* L. on seed germination and seedling growth of some cultivated and wild herbaceous species. *Scientific World,* **5**(5): 33-39.

Pimentel MR, Molina G, Dion´ısio AP, Junior MRM and Pastore GM (2011). The use of endophytes to obtain bioactive compounds and their application in biotransformation process. *Biotechnol. Res. Int.,* **1**: 1-11.

Riaz T and Javaid A (2009). Invasion of hostile alien weed *Parthenium hysterophorus* L. in Wah Cantt, *Pakistan. JAPS.,* **19**(1): 26-29.

Romero A, Carrion G and Rico Gray V (2001). Fungal latent pathogens and endophytes from leaves of *Parthenium hysterophorus* (Asteraceae). *Fungal Diversity,* **7**: 81-87.

Shabbir A and Javaid A (2010). Effect of aqueous extracts of alien weed *Parthenium hysterophorus* and two native asteraceous species on germination and growth of mungbean, *Vigna radiata* L. wilczek. *J. Agric. Res*., **48**(4): 483-488.

Shirling EB and Gottlieb D (1966). Methods for characterization of *Streptomyces* species. *Intl. J. Syst. Bacteriol.,* **16**: 313-340.

Silvani VA, Fracchia S, Fernández L, Pergola A and Godeas, A (2008). Short Communication. A simple method to obtain endophytic microorganisms from field-collected roots. *Soil. Biol. Biochem.,* **40**: 1259-1263.

Strobel G and Daisy B (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev.,* **67**(4): 491-502.

Strobel G, Daisy B, Castillo U and Harper J (2004). Natural products from endophytic microorganisms. *Harper J. Nat. Prod.,* **67**(2): 257-268.

Tamado T and Milberg P (2000). Weed flora in arable fields of eastern Ethiopia with emphasis on the occurrence of *Parthenium hysterophorus*. *Weed Research,* **40**: 507-521.

Zin NM, Sarmin NIM, Ghadin N, Basri DF, Sidik NM, Hess WM, Strobel GA (2007). Bioactive endophytic streptomycetes from the Malay Peninsula. *FEMS Microbiol Lett*., **274**: 83-88.